Intracranially Recorded Memory-related Potentials Reveal Higher Posterior than Anterior Hippocampal Involvement in Verbal Encoding and Retrieval

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Abstract

■ The human hippocampus is essential for both encoding and recollection, but it remains controversial whether there is a functionally different involvement of anterior versus posterior parts of the hippocampus in these memory processes. In the present study, we examined encoding and retrieval processes via intrahippocampal recordings in 27 patients with unilateral temporal lobe epilepsy. Multicontact depth electrodes were implanted along the longitudinal axis of the hippocampus as part of the presurgical evaluation. In a continuous word recognition test, subjects had to indicate whether words were new or already presented. Recognized old words, as compared to new words, resulted in a larger P600 component, as well as in a larger late negative component (LNC, 600–900 msec). In addition, subsequently remembered words elicited a larger positivity (400 to 900 msec) than later forgotten words. We found differences concerning the distribution along the hippocampus for the LNC old–new effect, reflecting successful retrieval, as well as for the subsequent memory effect, reflecting successful encoding. Both effects were larger the further posterior an electrode was located in the hippocampus. Findings are suggestive for a predominant posterior hippocampal involvement in both verbal encoding and retrieval. ■

INTRODUCTION

Since the famous case study of H.M., it is generally accepted that the hippocampus is critical for explicit memory in humans (Scoville & Milner, 1957). Hippocampal activity has been associated with both encoding and recollection processes (Squire, 1992), but within the hippocampus, a further specialization might exist. A functional separation with encoding in the anterior half and retrieval in the posterior half of the hippocampus was proposed by a meta-analysis of positron emission tomography (PET) memory studies (Lepage, Habib, & Tulving, 1998). In contrast to this concept, Greicius et al. (2003) suggested that the middle and posterior hippocampus is activated more strongly than the anterior hippocampus by both verbal encoding and retrieval. A predominantly posterior hippocampal involvement in encoding was also concluded from a review of functional magnetic resonance imaging (fMRI) studies (Schacter & Wagner, 1999).

The functional separation of anterior and posterior parts of the hippocampus in memory processes has remained an open issue as yet (see also Henson, 2005). Beside differences in study material and experimental paradigms, inconsistencies between fMRI findings might be related to the problem of susceptibility artifacts, which diminish the sensitivity of fMRI to detect an activation especially in the anterior medial-temporal lobe (Greicius et al., 2003; Ojemann et al., 1997).

In contrast to fMRI and PET, event-related potentials (ERPs) have a very high temporal resolution and give a direct estimation of neuronal activity, but it is not possible to measure hippocampal activity with scalp ERPs because the hippocampus is arranged cylindrically, forming a closed field (Klee & Rall, 1977). This problem is illustrated by the P300 component, which is reliably recorded in the hippocampus (Halgren et al., 1980). However, the scalp P300 is not affected by hippocampectomy (Johnson, 1988). Thus, the hippocampal P300 does not, or only to a minor degree, contribute to the P300 signal at the scalp.

Recordings with electrodes implanted in the hippocampus of patients with pharmacoresistant temporal lobe epilepsy (TLE) offer the rare opportunity to measure hippocampal activity directly, both with high temporal resolution and excellent signal-to-noise ratio. Previous studies with intracranial electrodes in the human hippocampus have shown that words elicit two different ERP components: the P600, which is a positivity between ~300 and 800 msec, and a late negative component (LNC), which is a negativity between ~600 and 1400 msec. Both components are larger for old than for new words and, therefore, are assumed to reflect memory processes (Grunwald et al., 2003; Smith, Stapleton, & Halgren, 1986). Usually, the P600 old–new effect is maximal between 300 and 600 msec (Grunwald et al., 2003). The intracranial P600 is labeled with reference to the surface

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nomenclature (Guillem, N'Kaoua, Rougier, & Claverie, 1995), but actually peaks around 450 msec in response to old words and around 550 msec in response to new words.

During encoding, the P600 amplitude to new words increased with successful memory formation (Fernández, Klaver, Fell, Grunwald, & Elger, 2002; Fernández et al., 1999). In contrast to the P600 old–new effect, this subsequent memory effect (with a more positive P600 to new words that are later remembered than for new words that are later forgotten) is a long-lasting effect measurable till up to 2000 msec after stimulus onset (Fernández et al., 1999, 2002). Beside its relevance for the encoding of verbal material, the P600 is also sensitive to general semantic association processes (Dietl et al., 2005; Klaver et al., 2005; Vannucci et al., 2003).

During encoding, there was no subsequent memory effect for the LNC (Fernández et al., 1999), but the LNC for successfully recognized old words differed significantly from the LNC for new words (Grunwald et al., 2003). This old–new effect on the LNC was larger in an intentional recognition task than in an incidental one (Grunwald et al., 2003). Thus, the LNC probably reflects successful retrieval processes. Although it could be assumed that P600 and LNC should be larger for verbal material on the left side and for pictorial material on the right side, no significant hemispheric differences have been found in previous studies (Klaver et al., 2005; Vannucci et al., 2003; Grunwald, Elger, Lehnertz, Van Roost, & Heinze, 1995).

Until now, no systematic evaluation of the spatial distribution of invasive ERP memory effects along the hippocampus axis has been undertaken, although such an analysis offers the possibility to dissociate encoding and retrieval processes with a high temporal and spatial resolution and to test previous hypotheses about a functional separation within the hippocampus. For example, if encoding is supported by the anterior, and retrieval by the posterior part of the hippocampus (Lepage et al., 1998), then the subsequent memory effect should be larger in the anterior hippocampus, and the LNC old–new effect larger in the posterior hippocampus. In the current study, we examined P600 and LNC old–new effects as well as the subsequent memory effects along the longitudinal axis of the hippocampus in order to assess functional separations within the hippocampus in memory processes. We further tested for hemispheric differences.

METHODS

Subjects

We investigated patients with pharmacoresistant unilateral TLE, who were implanted with bilateral depth electrodes along the longitudinal axis of the hippocampus during presurgical evaluation. Only recordings from the nonepileptic temporal lobe and only patients with at least one electrode located in the hippocampal head or three electrodes in the hippocampal body were included. Patients with demonstrated atypical language dominance were excluded, but language dominance (Wada test or fMRI) was not tested in all cases. All but one patient were right handed. In the left-handed patient, fMRI language testing indicated typical left hemispheric language lateralization. Thus, 27 patients (10 women; 13 left, 14 right TLE) were included in the study (see Table 1 for patient characteristics). Patients ranged in age from 18 to 61 years (mean = 42 years) and in duration of their epilepsy from 4 to 57 years (mean = 26 years). All participants had normal or corrected-to-normal vision. MRI scans or histological examinations demonstrated hippocampal sclerosis in 18 patients, extrahippocampal lesions without signs of hippocampal sclerosis in 8 patients, and no clear lesion in 1 patient. All but two patients underwent subsequent epilepsy surgery after implantation (16 selective amygdalohippocampectomies, 5 temporal two-thirds resections, 4 lesionectomies). The test paradigm is part of the routine presurgical workup in patients with hippocampal depth electrodes. The study was approved by the ethics committee of the University of Bonn.

Paradigms

For a continuous word recognition paradigm, 300 frequent German nouns were selected (mean word frequency was 50 per 1 million words according to the CELEX lexical database, version 2.5). One hundred fifty stimuli were only presented once, whereas the other 150 words were shown with one repetition. This repetition occurred in 50% of the trials after a short lag of 3 to 6 words and in 50% after a long lag of 10 to 30 words. In line with a previous study (Grunwald, Lehnertz, Heinze, Helmstaedter, & Elger, 1998), the analyses of our own ERP data separated for short and long lags did not reveal any essential impact of the applied lag on the reported effects. For clarity, only the analysis of ERPs for the both lags collapsed is provided. 450 words were presented consecutively with a duration of 300 msec per word. The length of the interstimulus interval was randomized around ~2000 msec, but was increased in adjustment of the subject abilities in some cases (on average, the interval was 2155 ± 416 msec long). After each word, subjects had to indicate by pressing one of two buttons whether it was new or already presented before. The study was conducted in a special unit for simultaneous video and electroencephalogram (EEG) monitoring with the patient sitting in an adjustable chair and facing a monitor approximately 80 to 100 cm away. The words were presented in white color on a black background with a height of ~1.5° and a width of ~3° to 9° visual angle, depending on word length. Recordings were occasionally repeated on the following day, if performance was bad or ERPs were contaminated by spikes or sharp waves.

Table 1. Patient Characteristics and Performance in the Continuous Word Recognition Paradigm as well as an Overview of Available Electrodes

Subject	*Nonfocal Side*	*Sex*	*Age*	*Duration of Epilepsy*	*Pathology of Focal Side*	*Available Electrodes*
1	L	m	37	33	HS	HH
2	L	f	46	37	HS	HH
3	R	m	45	25	HS	HH
4	R	f	57	30	HS	HH
5[a]	R	m	54	43	HS	HH
6	L	f	37	4	HS	HH, HB
7	L	f	40	38	HS	HH, HB
8	L	m	43	30	HS	HH, HB
9	L	m	46	20	HS	HH, HB
10	R	m	61	57	HS	HH, HB
11[b]	L	m	50	34	HS + temporal lobe dysplasia	HH, HB
12[b]	L	m	45	29	Parieto-occipital astrocytoma	HH, HB
13[a]	L	m	34	4	HS + temporal lobe tumor	HH, HB
14	L	f	41	14	HS	HB
15	L	m	42	34	HS	HB
16	R	m	38	13	HS	HB
17	R	m	40	35	HS	HB
18	R	f	44	43	HS	HB
19	R	f	28	24	Amygdala-ganglioglioma	HB
20	R	f	38	9	Temporal blurring of the gray–white matter junction	HB
21	R	m	22	10	No clear lesion	HB
22	R	m	55	9	Occipito-temporal cavernoma	HB
23	L	m	47	39	Temporal arteriovenous malformation	HB
24	R	m	57	17	Temporal necrosis	HB
25	R	f	25	18	Temporal blurring of the gray–white matter junction	HB
26[b]	L	m	38	26	HS	HB
27[b]	L	f	18	16	Temporal dysplasia	HB

R = right; L = left; m = male; f = female; HS = hippocampal sclerosis; HH = hippocampal head electrode; HB = hippocampal body electrode.

[a]Completely excluded.

[b]Excluded from subsequent memory analysis.

Explored Brain Structures and Recording Sites

ERPs were recorded from bilateral depth electrodes implanted stereotactically along the longitudinal axis of the hippocampus. Each catheter-like, 1-mm-thick depth electrode contained 10 cylindrical platinum electrodes of 2.5 mm every 4 mm. Ideally, the first 3 of these 10 electrodes are located in the rhinal cortex, the next one or two on the border to the amygdala and up to six along the longitudinal axis of the hippocampus. For each patient, the precise placement of electrode contacts within the hippocampus was verified by axial and coronal 2-mm-sliced T2-weighted and 3-mm-sliced fluid-attenuated inversion recovery MRIs routinely acquired after electrode implantation. There were variabilities in placement of the electrodes across patients in the anterior–posterior and also inferior–superior direction. Therefore, numbers of patients varied for different analyses. Electrodes were

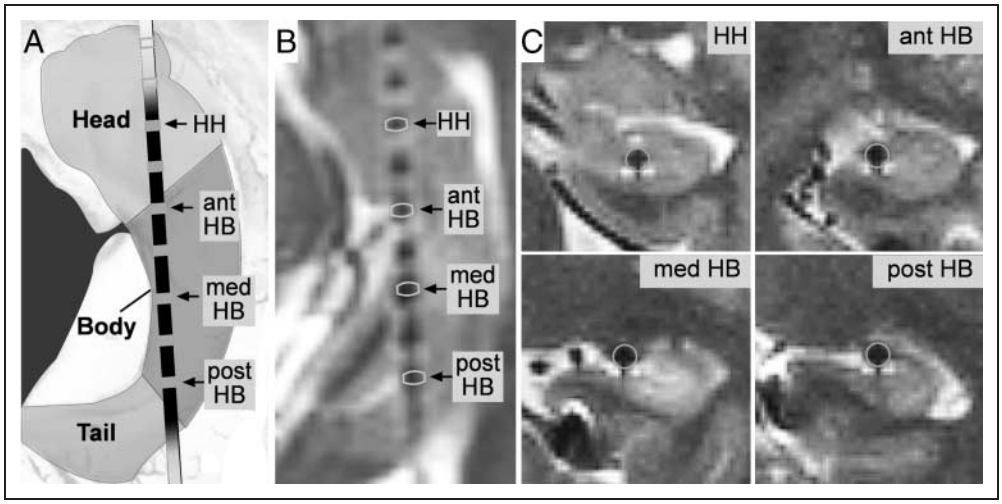

Figure 1. Location of hippocampal electrodes and exemplary MRI data (Patient 11). The selected hippocampal head electrode (HH), anterior hippocampal body electrode (ant HB), medial hippocampal body electrode (med HB), and posterior hippocampal body electrode (post HB): (A) in a schematic overview, (B) in the axial slice, (C) in coronal slices. The two electrodes visible in the axial slice anterior to HH are located in the amygdala.

grouped in hippocampal head and hippocampal body electrodes (Figure 1) according to the anatomy atlas of Duvernoy (1988). The first hippocampal body electrode was defined as the first position where the fimbria was visible and the uncus had disappeared.

Recordings

Electrophysiological data were recorded with the digital EPAS system (Schwarzer, Munich, Germany) and its implemented Harmonie EEG software (Stellate, Quebec, Canada). EEG was measured against left and right mastoids with a sampling rate of 200 or 1000 Hz. The 1000 Hz recorded data were resampled to 200 Hz. For the analysis of old–new effects, segments were averaged for correctly identified first presentations (''new'') and correctly identified repetitions (''old''). For the subsequent memory analysis, segments were averaged for first presentations, which were later successfully recognized (''rem''), and first presentations, which were later forgotten and not recognized when repeated (''forg'').

All EEG segments had a duration of 1700 msec with a 200-msec prestimulus period as baseline. Data were high-pass filtered at 0.1 Hz with a slope of 12 dB/octave and low-pass filtered at 12 Hz with a slope of 12 dB/octave. Amplitudes were measured relative to the mean amplitude of the 200-msec prestimulus baseline. An automated artifact rejection was implemented using Matlab 7.1 (Mathworks, Natick, MA). For each segment, the standard deviation of the data points as well as the standard deviation of the gradients (the increase or decrease between two successive data point) were determined. A segment was rejected if any data point or gradient deviated more than four standard deviations from the mean. Thus, segments with abnormally high amplitudes as well as abrupt rises or falls were eliminated. On average, 10.7% of trials were removed based on these criteria.

Electrode Selection

To study old–new as well as subsequent memory effects in different parts of the hippocampus, one electrode in the hippocampal head and three electrodes in the hippocampal body were selected. The hippocampal head (''HH'') electrode is defined as the most anterior electrode in the hippocampal head. In the hippocampal body, the most anterior (''ant HB''), the most posterior (''post HB''), and a medial hippocampal body electrode (''med HB'') were selected. The medial hippocampal body electrode was either the electrode that was located exactly in the middle of the anterior and posterior hippocampal body electrodes, or, if this was not possible, the mean average of two medial electrodes (see Figure 1 for an example of the anatomical location of contact labels).

Because the first electrodes were often located in the subiculum and not in the hippocampal head, there were less subjects with electrodes in the hippocampal head than with electrodes in the hippocampal body. In order to keep the number of subjects included in the analysis sufficiently high, the data of hippocampal head electrodes and of hippocampal body electrodes were analyzed separately.

Data Analysis

For the old–new effect, the mean amplitudes of three time windows were analyzed. The time window between 350 and 550 msec was chosen to measure the P600 old–new effect. We selected this time window because the P600 in response to new words shows a broader and shallower progression than for old words. Therefore, the P600 old–new effect is best measured in the time window before the steep P600 to old words crosses the smaller and shallower P600 to new words. The time windows between 600–900 msec and 900–1200 msec were chosen to measure the early and late LNC. To assess the subsequent memory effect, we analyzed the ''rem'' and

''forg'' trials in the time window between 400 and 900 msec. This longer time window as compared to the P600 old–new effect was selected because the subsequent memory effect covers later parts of the P600 as well.

For the evaluation of old–new or subsequent memory effects in the hippocampal head, we applied paired t tests. The number of patients with electrodes in the hippocampal head was too low for hemispheric evaluations (8 with left hemispheric and 3 with right hemispheric HH electrodes). For the hippocampal body, we were interested in differential memory effects along the hippocampal axis. For each memory effect (old–new, subsequent memory), separate analyses of variance (ANOVAs) were calculated.

Old–new effects were evaluated in a two-way repeated-measures ANOVA with old–new (old vs. new) and position (anterior, medial, posterior electrode in the hippocampal body) as within-subjects factors and hemisphere as a between-subject factor. An interaction between old–new effect and position would be expected if an old–new effect systematically varied along the longitudinal axis of the hippocampal body. For the analysis of subsequent memory effects, an analogue ANOVA was calculated with subsequent memory (rem vs. forg) and position as within-subject factors and hemisphere as a between-subject factor. For the direct comparison of hippocampal head and anterior hippocampal body electrodes, an ANOVA was calculated for the subgroup of patients who had electrodes in both regions, with position (hippocampal head vs. anterior hippocampal body) and old–new or subsequent memory as within-subject factors. The Greenhouse–Geisser correction was used when necessary, and is indicated by citation of ε values. Within the ANOVAs, the effects of position were tested for linearity. When significant effects were found, post hoc t tests for paired samples were applied.

RESULTS

Behavioral Data

On average, 86.6% (± 13.1) of the new words and 65.1% (± 22.7) of the old words were correctly categorized. This performance was significantly different from chance [new words: $t(26) = 34.407, p < .001$; old words: $t(26) = 14.917, p < .001$] and did not differ between patients with left and right focal hemisphere or between male and female patients (all t values < 0.700, *ns*). Also, reaction times for correct responses did not differ between new and old words [new words: 840 ± 192 msec; old words: 878 ± 156 msec, paired t test: $t(26) = 1.440$, *ns*].

ERPs in the Hippocampal Head

Old–New Effects

For the analysis of old–new effects, 11 patients with at least one electrode in the hippocampal head were included. Data for two other patients were excluded because the morphology of the ERPs was not comparable to the other patients. In the hippocampal head, the P600 elicited by old words was more positive than was the P600 to new words [$t(10) = 3.359, p < .01$]. The early LNC to old and new words did not differ [$t(10) = 0.239$, *ns*]. In the late LNC time window, new words were significantly more negative than old words [$t(10) = 3.923$, $p < .005$; Figure 2A, top panel].

Subsequent Memory Effect

For this analysis, two more patients were excluded because performance was extremely good, which left too few ''forgotten'' trials for a reliable calculation of ERPs. In the nine patients analyzed, no significant subsequent memory effect was observed in the hippocampal head [$t(8) = 1.671$, *ns*; Figure 2B, top].

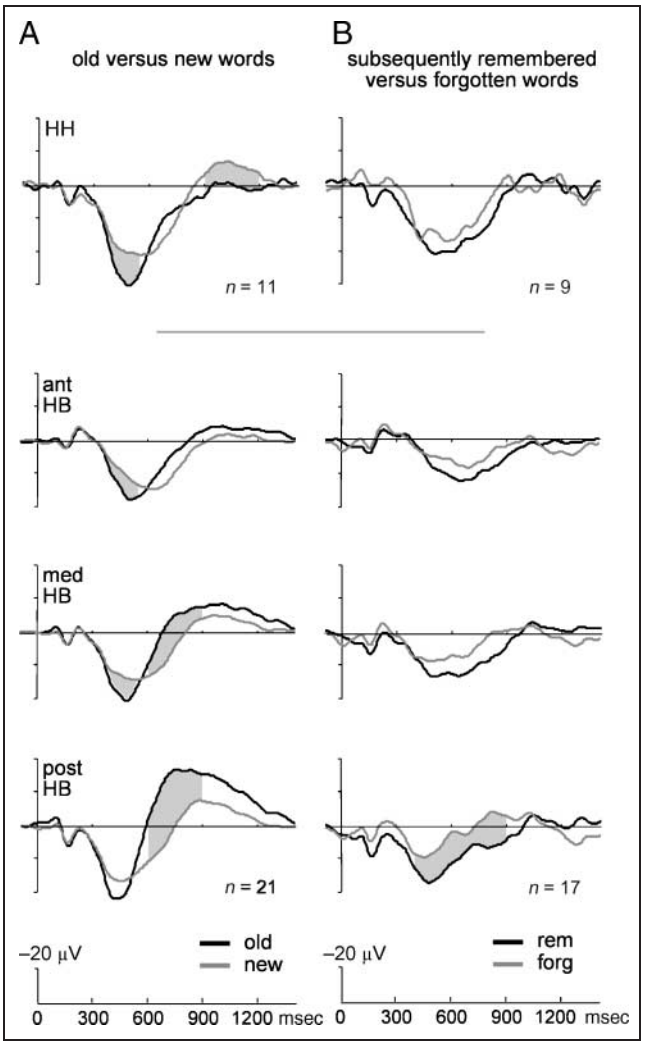

Figure 2. ERPs to (A) correctly recognized old versus new words and to (B) subsequently remembered (rem) versus forgotten (forg) words for hippocampal head (HH) and three hippocampal body (HB) electrodes. Significant differences in mean amplitude between old and new or later remembered and later forgotten are gray shaded. Negative values are plotted upward.

ERPs in the Hippocampal Body

Old–New Effects

For the analysis of old–new effects, 21 patients with at least three electrodes in the hippocampal body were included. Data of one patient were not evaluated because of unusual ERPs.

For the P600, the overall old–new effect reached significance [$F(1, 19) = 7.148$, $p < .05$], with larger P600 mean amplitudes for old than new words. No significant effects of position or interaction between position and old–new effect were observed (see Figure 2A for ERPs). In paired t tests for ERPs at each electrode separately, the old–new effects at the anterior and medial, but not at the posterior electrode, reached significance [anterior: $t(20) = 3.248, p < .005$; medial: $t(20) = 2.694, p < .05$; posterior: $t(20) = 1.610$, *ns*].

There was a more negative LNC for old words than for new words at all electrodes [$F(1, 19) = 7.158$, $p < .05$]. This old–new effect interacted with position, and was largest at the most posterior electrodes [linear effect: $F(1, 19) = 9.021$, $p < .01$], as also reflected in the p values of paired t tests at each of the three electrode positions [anterior: $t(20) = 1.662$, *ns*; medial: $t(20) = 2.199$, $p < .05$; posterior: $t(20) = 3.345$, $p < .005$; Figure 3A and C]. Independent of the old–new effect, mean amplitudes of the early LNC were overall largest at the most posterior electrodes in the hippocampal body [main effect of position: $F(1, 19) = 18.411$, $p < .001$]. This was also true for the late LNC [$F(1, 19) = 13.027$, $p < .005$], but there was no significant old–new effect or interaction between old–new effect and position in the late time window.

Subsequent Memory Effect

For the subsequent memory analysis, four patients were not included because they had forgotten too few words. Aside from a general subsequent memory effect with more positive amplitudes between 400 and 900 msec for subsequently remembered words at all electrodes [$F(1,$

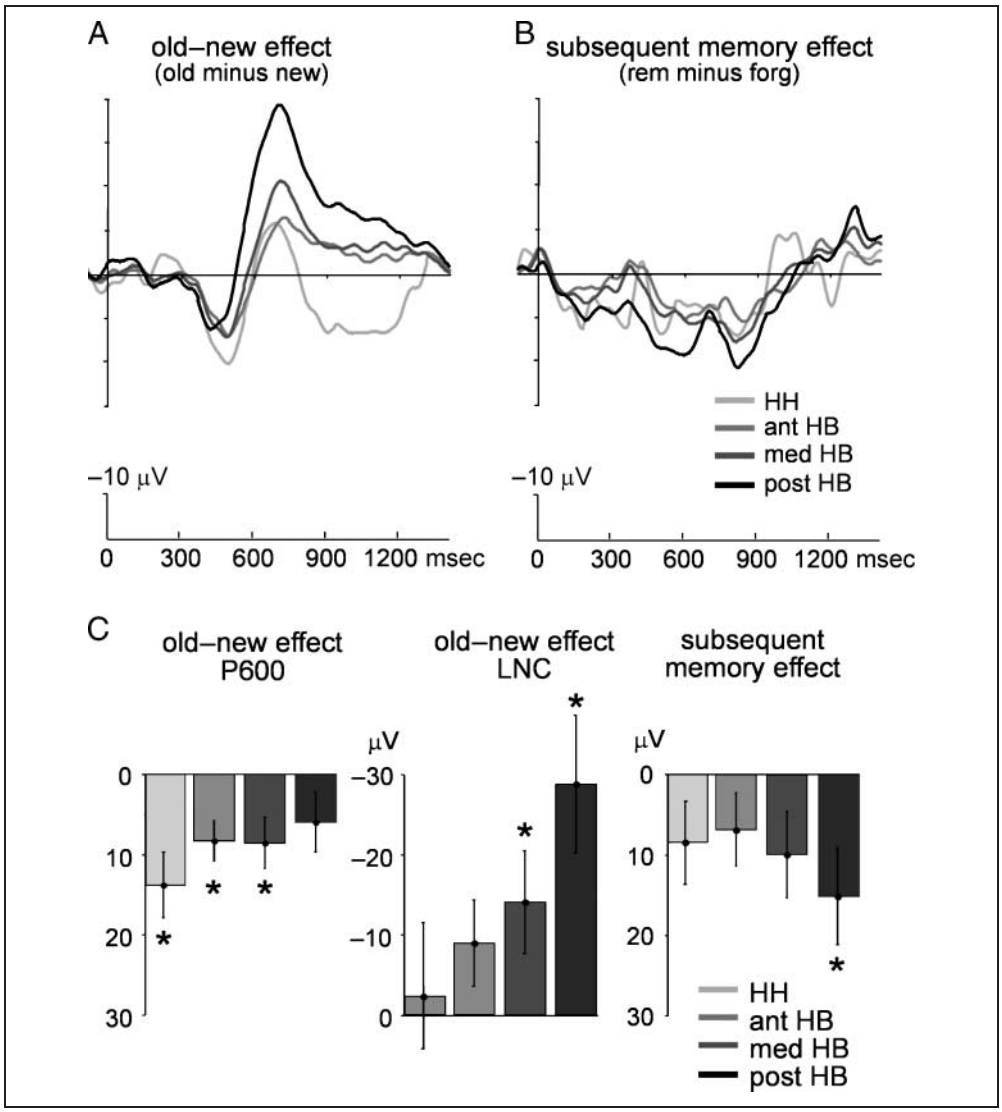

Figure 3. ERP difference waves (A) old minus new, (B) subsequently remembered minus forgotten, (C) the mean differences reflecting the P600 old–new effect, the early LNC old–new effect as well as the subsequent memory effect for hippocampal head (HH), anterior (ant HB), medial (med HB), and posterior (post HB) hippocampal body. Negative values are plotted upward.

15) = 4.729, $p < .05$], we also observed a linear increase of the subsequent memory effect with a more posterior position [$F(1, 15) = 4.541$, $p = .05$; Figure 3B]. Post hoc tests showed that there was no subsequent memory effect for the anterior and medial hippocampal body [$t(16) = 1.532$, *ns* and $t(16) = 1.890$, *ns*], but there was one for the posterior hippocampal body [$t(16) = 2.562$, $p < .05$; Figure 3C].

Hemispheric Differences

A significant effect of hemisphere was only observed for the P600 [$F(1, 19) = 7.896$, $p < .05$; Figure 4], which was larger on the left than right side. No interactions of hemisphere with old–new (Figure 4A) or subsequent memory effect (Figure 4B), and no interaction of hemisphere with position, were found for any ERP component.

Hippocampal Head versus Anterior Hippocampal Body

For all patients with hippocampal head electrodes, an anterior hippocampal body electrode was also available. Eleven patients were included in the old–new analysis and nine patients were included in the subsequent memory analysis. Concerning the P600 and LNC old–new data, no significant effects of position or interactions between position and old–new effect were observed [$F(1, 10) < 0.9$]. Of note, the old–new effect of the P600 was virtually the same at both electrodes for those patients (HH: 13.9 μV ± 13.7; anterior HB: 14.8 μV ± 14.2). Also, the subsequent memory effect did not differ significantly between the hippocampal head and anterior hippocampal body electrode [interaction $F(1, 8) < 1.2$, *ns*].

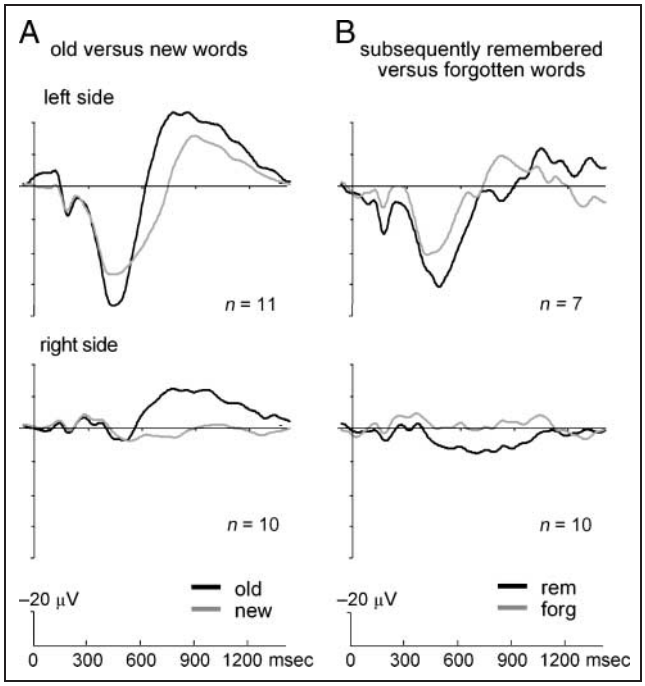

Figure 4. Left versus right hemisphere: (A) ERPs for correctly recognized old versus new words for the posterior hippocampal body electrode, (B) ERPs for subsequently remembered versus forgotten words for the posterior hippocampal body electrode. Negative values are plotted upward.

DISCUSSION

The aim of the present study was to evaluate systematically the distribution of old–new and subsequent memory effects along the longitudinal axis of the hippocampus. We found a linear increase for the early LNC old–new effect, as well as for the subsequent memory effect: Both effects were larger the further posterior the electrode was located in the hippocampus. The P600 old–new effect was equally significant in the hippocampal head and body. Hemispheric differences were observed for the P600, which was significantly larger on the left as compared to the right side; the old–new and subsequent memory effect did not differ between hemispheres. For the late LNC we found an inverted old–new (''novelty'') effect in the hippocampal head.

Retrieval and the Hippocampus

The comparison between words that were correctly recognized as new words and words that were correctly recognized as old words (''old–new effect'') can be used to explore successful recognition. The LNC old–new effect between 600 and 900 msec was assumed to reflect successful retrieval processes (Grunwald et al., 2003). In this time window (the ''early'' LNC), we observed a linear increase of the old–new effect along the hippocampal axis—with no old–new effect in the hippocampal head and anterior hippocampal body, but moderately sized effects in the medial and large effect in the posterior hippocampal body.

Our finding is in line with the majority of fMRI and PET studies showing that the posterior hippocampus is more involved in memory retrieval than its more anterior parts (Daselaar, Fleck, & Cabeza, 2006; Stark & Squire, 2000; Henson, Rugg, Shallice, Josephs, & Dolan, 1999; Lepage et al., 1998). To our knowledge, there are no studies showing a predominantly anterior hippocampal involvement during retrieval of single items. The anterior hippocampus might be more important in relational memory, as Giovanello, Schnyer, and Verfaellie (2004) found greater anterior hippocampal activity during retrieval of associations than during retrieval of single items.

As we did not collect confidence ratings in the behavioral task, we were not able to separate decisions based on recollection from those based on familiarity. Many studies propose that recollection activates the hippocampus more than does familiarity (Daselaar et al., 2006; Yonelinas, Otten, Shaw, & Rugg, 2005; Strange, Fletcher,

Henson, Friston, & Dolan, 1999). However, we were able to show strong hippocampal activity in the present study. If we intermixed recollection-based trials (with high activity) with familiarity-based trials and correct responses by chance (with smaller or no retrieval related activity), this might have affected the overall signal, but should not change the interpretation of differences along the longitudinal axis of the hippocampus.

Encoding and the Hippocampus

A suitable method to probe successful encoding is a comparison between stimuli that are later remembered and those that are later forgotten. In the hippocampus, subsequently recalled words are accompanied by a more positive ERP component than subsequently forgotten words (Fernández et al., 1999, 2002). As for the retrieval success-related LNC, we also found a linear increase of this subsequent memory effect along the hippocampal body. Thus, we assume that successful encoding is also subserved by the posterior part of the hippocampal body.

This assumption is in line with several event-related fMRI studies showing that posterior parts of the hippocampus exhibited larger activity than anterior parts for subsequently remembered words (Greicius et al., 2003; Reber, Siwiec, et al., 2002; Fernández et al., 1998). However, at least one event-related study claimed a predominant involvement of the anterior hippocampus in encoding (Kensinger, Clarke, & Corkin, 2003). Aside from these event-related fMRI studies specifically testing successful encoding, many other fMRI studies examined encoding in blocked designs, thus comparing blocks with an encoding task to blocks with a control task. Here, most of these studies also indicated a posterior focus for encoding (see Henson, 2005; Schacter & Wagner, 1999 for reviews), but especially PET studies often reported anterior hippocampal activity during encoding (Lepage et al., 1998). It remains unclear whether these PET and fMRI inconsistencies are based on differences in methodology (e.g., fMRI susceptibility artifacts can result in a loss of anterior hippocampal blood oxygenation level dependent signal; Greicius et al., 2003; Ojemann et al., 1997), or on differences in behavioral procedure (see Schacter & Wagner, 1999 for review). Generally, the use of blocked designs is somewhat problematic as encoding is analyzed without considering the success of encoding. Thus, effects of novelty detection, semantic encoding processes, and actual successful encoding are intermixed in these designs.

Furthermore, in most studies, analyses were not designed to directly compare anterior and posterior hippocampal activation, which would require a direct region-of-interest-based comparison (Constable et al., 1998). By applying such a region-of-interest-based comparison, Greicius et al. (2003) observed larger posterior than anterior hippocampal activation in their event-related design. Reber, Wong, and Buxton (2002) reported a similar finding, but in their study the difference did not reach significance, possibly due to the small sample size ($n = 5$).

Cameron, Yashar, Wilson, and Fried (2001) investigated successful encoding in an associative learning task by recordings from microelectrodes in the hippocampus. The authors reported that neurons in the anterior hippocampus responded selectively to the successful learning of associations. Unfortunately, their study design did not allow a comparison of anterior and posterior electrodes, and the authors did not specify how they defined the anteriority of electrodes. However, even the finding of encoding sensitive neurons in anterior regions of the hippocampus is not necessarily contradictory to our results. The increasing subsequent memory effect along the hippocampus found in our study might reflect an increasing number of neurons along the hippocampus axis involved in successful encoding. Another possibility is that associative learning results in more anterior hippocampal activation, whereas single-item learning is mediated more by the posterior hippocampus, as proposed by Schacter and Wagner (1999).

Semantic Association Processes and the Hippocampus

Aside from the subsequent memory effect, we also observed a P600 old–new effect reflecting larger activation in response to old than new words in hippocampal head and body. Thus, the P600 is influenced by two spatially and temporally distinguishable effects: By successful encoding with larger effects in the posterior than in the anterior hippocampal body, and by a short-lasting effect of word repetition, with an equal distribution in the hippocampal head and body.

Encoding is an important feature of the hippocampus, but before a stimulus can be encoded, it must be activated in the semantic lexicon, which is likely mediated by the frontal lobes (Fletcher, Shallice, & Dolan, 2000; Poldrack et al., 1999). Addis and McAndrews (2006) presented triads of words with either no, two, or all three words being semantically associated. The authors observed larger inferior frontal cortex activity when less associations were provided and larger left hippocampal activity when more associations were provided. Thus, it was supposed that the inferior frontal cortex is responsible for providing semantic associations, whereas the hippocampus binds the provided associations during encoding.

We propose that the P600 represents an index of these provided associations. This would be in line with the previous findings, revealing a larger P600 for real than unreal objects (Vannucci et al., 2003), for famous than nonfamous faces (Dietl et al., 2005), and for concrete words with high imageability than for abstract words (Klaver et al., 2005). If a word was previously encoded, the associations are more easily reactivated and more associations are accessible to the hippocampus,

explaining a larger P600 for old words (Grunwald et al., 2003). Because the P600 old–new effect was not significantly different between hippocampal head and anterior hippocampal body or along the hippocampal body, these semantic association processes might be mediated by extended hippocampal networks.

Hemispheric Differences

The mean amplitudes of the P600 between 350 and 550 msec were significantly larger on the left than on the right side for new and old words, whereas the left–right comparison for new words between 400 and 900 msec was not significant. There were no significant differences concerning old–new effects or the subsequent memory effect. This suggests that the left hemisphere is more involved in semantic association processes than the right hemisphere, but that both hemispheres are equally involved in memory encoding and retrieval.

The larger involvement of the left hippocampus in association processes is in line with the Addis and McAndrews (2006) study, where also larger left hippocampal activity was observed when more associations were provided in a word association learning task. However, previous studies did not find hemispheric differences of the P600 in the continuous word recognition paradigm (Klaver et al., 2005; Grunwald et al., 1995) or in an object recognition task (Vannucci et al., 2003).

Several studies reported left hemispheric activity to word stimuli during encoding (Strange, Otten, Josephs, Rugg, & Dolan, 2002) or retrieval (Fliessbach, Weis, Klaver, Elger, & Weber, 2006; Eldridge, Knowlton, Furmanski, Bookheimer, & Engel, 2000), underlining the dominant role of the left hemisphere in verbal processing. In the current study, the LNC old–new effect reflecting successful retrieval, as well as the subsequent memory effect reflecting successful encoding, was observable to a comparable extent on the left and right sides. Other studies revealed also bilateral hippocampal activity during encoding (Cameron et al., 2001; Fernández et al., 1998) and retrieval of verbal material (Yonelinas et al., 2005). Beside other factors, it might depend on task characteristics, whether the language-dominant hemisphere exhibits a larger hippocampal activation during a verbal memory task or not. Overall, both the lack of hemispheric differences in memory functions in the current study, as well as the found hemispheric differences, should be interpreted with some caution, as the left and right hemispheric ERPs were obtained in different subjects.

Novelty and the Hippocampus

In the hippocampal head, a late negativity (''the late LNC'') was shown to be larger for new than old words, thus reflecting a novelty effect. This finding of an ''inverted'' LNC old–new effect was unexpected and not shown with intracranial recordings before. However, it is supported by various fMRI studies (Daselaar et al., 2006; Strange & Dolan, 2001; Strange et al., 1999; Dolan & Fletcher, 1997; Tulving, Markowitsch, Craik, Habib, & Houle, 1996) that especially anterior regions of the hippocampus are sensitive to stimulus novelty. In this context, it should be noted that the N400 component, recorded in the rhinal cortex, is also larger for new than old words between 300 and 600 msec, and thus, also sensitive to novelty (Grunwald et al., 1995). A role of the hippocampus in novelty detection is in line with the finding that the integrity of the hippocampus is critical for the novelty detection in the rhinal cortex. For instance, hippocampal sclerosis decreases the rhinal N400 to new but not to old words (Grunwald et al., 1998) and the rhinal N400 to new words is correlated with neuronal density in the CA1 subfield of the hippocampus (Grunwald et al., 1999).

Local Generation of the ERPs

The LNC and P600 are most likely generated locally in the hippocampus. Previous studies have shown that the distribution and polarity of the P600 varied greatly between MTL structures such as the amygdala, the hippocampus, and the parahippocampal gyrus, and that the polarity inverted over short distances within the hippocampus (Smith et al., 1986). This suggests that the potentials are generated by local synapses and do not reflect activity from distant sources (Halgren et al., 1980). Although we did not observe polarity inversions within the hippocampus, both the P600 and LNC were detectable within the hippocampus, but not immediately outside of it: neither in the rhinal or parahippocampal cortex, nor in the amygdala or posterior to the hippocampus. Neurons in the hippocampus are arranged cylindrically (Amaral & Insausti, 1990), producing a radially symmetric closed field (Klee & Rall, 1977). Hippocampal activity is thus shielded toward the outside, and the LNC component, as well as the P600, are most likely generated locally (Fernández et al., 2002). Furthermore, due to the laminated structure of the hippocampus, synaptic current flows in the hippocampus tend to summate rather than to cancel out, providing a basis for large hippocampal ERPs (Smith et al., 1986).

Limitations of the Study

For clinical considerations, the exact location and number of the electrodes in the hippocampus is of minor relevance. This variability of electrode location has some impact on our study design and the interpretation of the data: (1) Only a low number of patients had electrodes in the hippocampal head *and* more than two electrodes in the hippocampal body. Thus, a statistical comparison of ERPs obtained at the hippocampal head and body was possible only in a subset of patients and, therefore, was statistically less sensitive than the analysis within the

hippocampal body. However, the results of both analyses did not conflict in any aspect. Thus, there is no reason to assume that the inclusion of a larger number of patients with hippocampal head electrodes would have affected our major findings. (2) The hippocampal head electrodes as well as the anterior hippocampal body electrodes were chosen strictly according to the MRI images. In contrast, the medial and posterior hippocampal body electrodes were defined based on their relative positions because the number of electrodes in the hippocampus body considerably varied in our sample. We decided to include all subjects with three to five hippocampal body electrodes in order to include as many subjects as possible, and thus, to provide a data basis, as reliable as possible. The inclusion of a larger number of patients with five electrodes in the hippocampus body might have resulted in more pronounced findings, but would have extended the temporal scope of the study tremendously. (3) The hippocampal tail is extremely rarely penetrated by electrodes. Therefore, it was not possible to study the functions of this region.

Other factors might have also influenced our findings. All our subjects were epileptic patients, and it is yet difficult to assess the impact of the epileptic focus on brain functions. However, it appears rather unlikely that the epileptic focus affects the component localization along the hippocampus of the nonfocal hemisphere. Epilepsy should also be taken into account for the interpretation of hemispheric differences, as the hemispheric dominance in epilepsy patients might be affected by cortical plasticity and secondary effects, as for instance, crowding (Helmstaedter, Brosch, Kurthen, & Elger, 2004; Strauss, Satz, & Wada, 1990). As already outlined, the current findings concerning hemispheric differences are limited by the fact that it was not possible to compare left and right hemispheres of the same subjects (because, in each case, one hemisphere was affected by the epileptic focus).

Finally, in the continuous word recognition paradigm, items have to be encoded and retrieved at the same time. Although the chosen contrasts (old–new effect as reflecting successful retrieval, and subsequent memory effect as reflecting successful encoding) theoretically correct for this impreciseness, a task with separate phases of encoding and retrieval might be better suited to dissociate these two functions.

Conclusion: Memory in the Medial Temporal Lobe

In a continuous word recognition paradigm, ERP effects previously associated with successful encoding and successful retrieval were larger more posterior in the hippocampal body. Therefore, we assume that similar posterior hippocampal neuronal networks are activated during encoding and retrieval of single items. ERP effects likely reflecting novelty detection were more pronounced in the hippocampal head, whereas effects presumably reflecting semantic association processes did not differ between anterior and posterior parts. Thus, although novelty detection, semantic association processes, and successful encoding are closely related processes (Strange, Hurlemann, Duggins, Heinze, & Dolan, 2005), the results of our study suggest a regional dissociation.

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REFERENCES

Addis, D. R., & McAndrews, M. P. (2006). Prefrontal and hippocampal contributions to the generation and binding of semantic associations during successful encoding. *Neuroimage, 33,* 1194–1206.

Amaral, D. G., & Insausti, R. (1990). Hippocampal formation. In G. Paxinos (Ed.), *The human nervous system* (pp. 711–755). San Diego, CA: Academic Press.

Cameron, K. A., Yashar, S., Wilson, C. L., & Fried, I. (2001). Human hippocampal neurons predict how well word pairs will be remembered. *Neuron, 30,* 289–298.

Constable, R. T., Skudlarski, P., Mencl, E., Pugh, K. R., Fulbright, R. K., Lacadie, C., et al. (1998). Quantifying and comparing region-of-interest activation patterns in functional brain MR imaging: Methodology considerations. *Magnetic Resonance Imaging, 16,* 289–300.

Daselaar, S. M., Fleck, M. S., & Cabeza, R. (2006). Triple dissociation in the medial temporal lobes: Recollection, familiarity, and novelty. *Journal of Neurophysiology, 96,* 1902–1911.

Dietl, T., Trautner, P., Staedtgen, M., Vannuchi, M., Mecklinger, A., Grunwald, T., et al. (2005). Processing of famous faces and medial temporal lobe event-related potentials: A depth electrode study. *Neuroimage, 25,* 401–407.

Dolan, R. J., & Fletcher, P. C. (1997). Dissociating prefrontal and hippocampal function in episodic memory encoding. *Nature, 388,* 582–585.

Duvernoy, H. M. (1988). The human hippocampus. *An atlas of applied anatomy* (pp. 25–43). München: J. F. Bergmann Verlag.

Eldridge, L. L., Knowlton, B. J., Furmanski, C. S., Bookheimer, S. Y., & Engel, S. A. (2000). Remembering episodes: A selective role for the hippocampus during retrieval. *Nature Neuroscience, 3,* 1149–1152.

Fernández, G., Effern, A., Grunwald, T., Pezer, N., Lehnertz, K., Dumpelmann, M., et al. (1999). Real-time tracking of memory formation in the human rhinal cortex and hippocampus. *Science, 285,* 1582–1585.

Fernández, G., Klaver, P., Fell, J., Grunwald, T., & Elger, C. E. (2002). Human declarative memory formation: Segregating rhinal and hippocampal contributions. *Hippocampus, 12,* 514–519.

Fernández, G., Weyerts, H., Schrader-Bolsche, M., Tendolkar, I., Smid, H. G., Tempelmann, C., et al. (1998). Successful verbal encoding into episodic memory engages the posterior

hippocampus: A parametrically analyzed functional magnetic resonance imaging study. *Journal of Neuroscience, 18,* 1841–1847.
Fletcher, P. C., Shallice, T., & Dolan, R. J. (2000). ''Sculpting the response space''—An account of left prefrontal activation at encoding. *Neuroimage, 12,* 404–417.
Fliessbach, K., Weis, S., Klaver, P., Elger, C. E., & Weber, B. (2006). The effect of word concreteness on recognition memory. *Neuroimage, 32,* 1413–1421.
Giovanello, K. S., Schnyer, D. M., & Verfaellie, M. (2004). A critical role for the anterior hippocampus in relational memory: Evidence from an fMRI study comparing associative and item recognition. *Hippocampus, 14,* 5–8.
Greicius, M. D., Krasnow, B., Boyett-Anderson, J. M., Eliez, S., Schatzberg, A. F., Reiss, A. L., et al. (2003). Regional analysis of hippocampal activation during memory encoding and retrieval: fMRI study. *Hippocampus, 13,* 164–174.
Grunwald, T., Beck, H., Lehnertz, K., Blumcke, I., Pezer, N., Kurthen, M., et al. (1999). Evidence relating human verbal memory to hippocampal *N*-methyl-D-aspartate receptors. *Proceedings of the National Academy of Sciences, U.S.A., 96,* 12085–12089.
Grunwald, T., Elger, C. E., Lehnertz, K., Van Roost, D., & Heinze, H. J. (1995). Alterations of intrahippocampal cognitive potentials in temporal lobe epilepsy. *Electroencephalography and Clinical Neurophysiology, 95,* 53–62.
Grunwald, T., Lehnertz, K., Heinze, H. J., Helmstaedter, C., & Elger, C. E. (1998). Verbal novelty detection within the human hippocampus proper. *Proceedings of the National Academy of Sciences, U.S.A., 95,* 3193–3197.
Grunwald, T., Pezer, N., Munte, T. F., Kurthen, M., Lehnertz, K., Van Roost, D., et al. (2003). Dissecting out conscious and unconscious memory (sub)processes within the human medial temporal lobe. *Neuroimage, 20*(Suppl. 1), S139–S145.
Guillem, F., N'Kaoua, B., Rougier, A., & Claverie, B. (1995). Intracranial topography of event-related potentials (N400/P600) elicited during a continuous recognition memory task. *Psychophysiology, 32,* 382–392.
Halgren, E., Squires, N. K., Wilson, C. L., Rohrbaugh, J. W., Babb, T. L., & Crandall, P. H. (1980). Endogenous potentials generated in the human hippocampal formation and amygdala by infrequent events. *Science, 210,* 803–805.
Helmstaedter, C., Brosch, T., Kurthen, M., & Elger, C. E. (2004). The impact of sex and language dominance on material-specific memory before and after left temporal lobe surgery. *Brain, 127,* 1518–1525.
Henson, R. (2005). A mini-review of fMRI studies of human medial temporal lobe activity associated with recognition memory. *Quarterly Journal of Experimental Psychology B, 58,* 340–360.
Henson, R. N., Rugg, M. D., Shallice, T., Josephs, O., & Dolan, R. J. (1999). Recollection and familiarity in recognition memory: An event-related functional magnetic resonance imaging study. *Journal of Neuroscience, 19,* 3962–3972.
Johnson, R. (1988). Scalp-recorded P300 activity in patients following unilateral temporal lobectomy. *Brain, 111,* 1517–1529.
Kensinger, E. A., Clarke, R. J., & Corkin, S. (2003). What neural correlates underlie successful encoding and retrieval? A functional magnetic resonance imaging study using a divided attention paradigm. *Journal of Neuroscience, 23,* 2407–2415.
Klaver, P., Fell, J., Dietl, T., Schur, S., Schaller, C., Elger, C. E., et al. (2005). Word imageability affects the hippocampus in recognition memory. *Hippocampus, 15,* 704–712.
Klee, M., & Rall, W. (1977). Computed potentials of cortically arranged populations of neurons. *Journal of Neurophysiology, 40,* 647–666.
Lepage, M., Habib, R., & Tulving, E. (1998). Hippocampal PET activations of memory encoding and retrieval: The HIPER model. *Hippocampus, 8,* 313–322.
Ojemann, J. G., Akbudak, E., Snyder, A. Z., McKinstry, R. C., Raichle, M. E., & Conturo, T. E. (1997). Anatomic localization and quantitative analysis of gradient refocused echo-planar fMRI susceptibility artifacts. *Neuroimage, 6,* 156–167.
Poldrack, R. A., Wagner, A. D., Prull, M. W., Desmond, J. E., Glover, G. H., & Gabrieli, J. D. (1999). Functional specialization for semantic and phonological processing in the left inferior prefrontal cortex. *Neuroimage, 10,* 15–35.
Reber, P. J., Siwiec, R. M., Gitelman, D. R., Parrish, T. B., Mesulam, M. M., & Paller, K. A. (2002). Neural correlates of successful encoding identified using functional magnetic resonance imaging. *Journal of Neuroscience, 22,* 9541–9548.
Reber, P. J., Wong, E. C., & Buxton, R. B. (2002). Encoding activity in the medial temporal lobe examined with anatomically constrained fMRI analysis. *Hippocampus, 12,* 363–376.
Schacter, D. L., & Wagner, A. D. (1999). Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. *Hippocampus, 9,* 7–24.
Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery and Psychiatry, 20,* 11–21.
Smith, M. E., Stapleton, J. M., & Halgren, E. (1986). Human medial temporal lobe potentials evoked in memory and language tasks. *Electroencephalography and Clinical Neurophysiology, 63,* 145–159.
Squire, L. R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. *Psychological Review, 99,* 195–231.
Stark, C. E., & Squire, L. R. (2000). Functional magnetic resonance imaging (fMRI) activity in the hippocampal region during recognition memory. *Journal of Neuroscience, 20,* 7776–7781.
Strange, B. A., & Dolan, R. J. (2001). Adaptive anterior hippocampal responses to oddball stimuli. *Hippocampus, 11,* 690–698.
Strange, B. A., Fletcher, P. C., Henson, R. N., Friston, K. J., & Dolan, R. J. (1999). Segregating the functions of human hippocampus. *Proceedings of the National Academy of Sciences, U.S.A., 96,* 4034–4039.
Strange, B. A., Hurlemann, R., Duggins, A., Heinze, H. J., & Dolan, R. J. (2005). Dissociating intentional learning from relative novelty responses in the medial temporal lobe. *Neuroimage, 25,* 51–62.
Strange, B. A., Otten, L. J., Josephs, O., Rugg, M. D., & Dolan, R. J. (2002). Dissociable human perirhinal, hippocampal, and parahippocampal roles during verbal encoding. *Journal of Neuroscience, 22,* 523–528.
Strauss, E., Satz, P., & Wada, J. (1990). An examination of the crowding hypothesis in epileptic patients who have undergone the carotid amytal test. *Neuropsychologia, 28,* 1221–1227.
Tulving, E., Markowitsch, H. J., Craik, F. E., Habib, R., & Houle, S. (1996). Novelty and familiarity activations in PET studies of memory encoding and retrieval. *Cerebral Cortex, 6,* 71–79.
Vannucci, M., Dietl, T., Pezer, N., Viggiano, M. P., Helmstaedter, C., Schaller, C., et al. (2003). Hippocampal function and visual object processing in temporal lobe epilepsy. *NeuroReport, 14,* 1489–1492.
Yonelinas, A. P., Otten, L. J., Shaw, K. N., & Rugg, M. D. (2005). Separating the brain regions involved in recollection and familiarity in recognition memory. *Journal of Neuroscience, 25,* 3002–3008.